

Recombinant Human Lactoferrin to Reduce Immune Activation and Coagulation Among HIV Positive Patients: *A pilot study*

**Short Title: rh-Lactoferrin-HIV Study
[PCC-006]**

ClinicalTrials.gov Identifier: NCT01830595

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(Ventria Bioscience *referenced* IND Number: 111060)

A Positive Care Center (PCC) single-site clinical trial conducted at HCMC

HCMC Human Subjects Research Committee #13-3657

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NOTE: This protocol was developed consistent with 'Standard Protocol Items: Recommendations for Intervention Trials' (the SPIRIT initiative).¹

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1. INTRODUCTION

1.1. Background and Rationale

The Changing Spectrum of Morbidity and Mortality Among HIV Positive Patients:

Antiretroviral therapy (**ART**) effectively and durably suppresses HIV replication, leads to immune recovery (increasing CD4+ T-cell counts), prolonged life expectancy, and has fundamentally changed the spectrum of morbidity and mortality among HIV positive persons.²⁻⁸ Among well-treated patients with levels of HIV RNA below the level of detection, non-AIDS-related conditions are now a more common cause of morbidity and mortality than AIDS.^{6, 9-11} The most relevant serious non-AIDS-related diseases in current clinical practice include atherosclerotic cardiovascular disease (CVD), cancer, liver disease, end-stage renal disease, bone disease and subclinical neurocognitive dysfunction. Of these, CVD and cancer constitute the vast majority of clinical events.^{5, 10-13}

Excess risk for serious non-AIDS-related conditions among HIV positive persons is due to multiple factors, including a higher burden of traditional risk factors, ART toxicity and chronic inflammation. Recent data from a number of well-conducted epidemiologic studies have shown that key biomarkers of inflammation and coagulation and markers cellular activation—all of which improve with ART but do not normalize to uninfected population levels—predict risk for CVD, cancer, and mortality.¹⁴⁻²⁰ Levels of two such markers, D-dimer and interleukin-6 (IL-6), were strongly associated with CVD and all-cause mortality in the Strategic Management of AntiRetroviral Therapy study (**SMART**; OR for 4th/1st quartile were 2.4 and 26.5 for D-dimer, and 3.5 and 11.8 for IL-6, respectively).¹⁴ We have built on these observations and measured IL-6 and D-dimer levels among participants from 3 separate trials (conducted by 'INSIGHT' network). The resulting cohort consists of 3,766 participants receiving continuous ART for up to 8 years of follow-up. In this cohort, IL-6 and D-dimer levels remain strongly associated with risk for CVD and all-cause mortality, and IL-6 levels also predict cancer risk.^{21, 22}

Factors Contributing to Ongoing Immune Activation:

Immune dysfunction, activation of lymphocytes, and elevated levels of inflammatory cytokines are hallmarks of untreated HIV infection.^{14, 23} The level of T-cell activation (e.g., CD38/HLA-DR expression), reflecting adaptive immunity, predicts risk for AIDS²⁴ and has been associated with CVD risk in cross-sectional studies.^{25, 26} More recent data demonstrate that innate immune activation also contributes to chronic inflammation.²⁷⁻²⁹ Monocytes that are functionally more pro-inflammatory (i.e., CD14+/CD16+, referred to as an intermediate subtype) or exhibit greater affinity for vascular surfaces (i.e., CD14^{dim}/CD16+, or non-classical subtype) those expressing CD16+) are more frequent with HIV infection,³⁰⁻³³ and the CD16+ monocyte subpopulations in particular may be preferentially infected by HIV.³⁴ Finally, we found that higher frequencies of CD14+/CD16+ monocytes predict greater progression of coronary artery calcium among 436 HIV positive patients, independent of traditional CVD risk factors, CD4 count and viral load.³⁵ These, and other, cellular activation phenotypes will be important secondary outcomes, to explore possible mechanisms for observed treatment effects on IL-6 and D-dimer.

The precise mechanisms driving high level immune activation are not entirely clear, but appear to involve both a persistent anti-HIV response (even with HIV RNA at low levels) and a more generalized immune activation (e.g., cytokine release).^{19, 36-38} Specifically, innocent bystander activation—in which the pro-inflammatory aspects of HIV infection non-specifically drive immune activation—also contributes.^{37, 39, 40} Factors specific to HIV infection that may account for excess inflammation also include: a) HIV-mediated destruction of gut epithelium, which leads to chronic

translocation of bacterial products, b) dysregulated inflammatory responses to co-pathogens (e.g., CMV), c) HIV-persistence, d) metabolic abnormalities, and d) loss of key regulatory cells (i.e., T-regulatory cells).⁴¹⁻⁴⁸

Rationale for Lactoferrin Treatment to Reduce Inflammation:

In the context of HIV infection, treatments strategies could target the well-characterized mechanisms driving persistent inflammation in HIV+ persons such as microbial translocation, residual HIV replication, and high co-pathogen burdens (e.g., CMV, HCV). In contrast, treatments that target the inflammatory or coagulation response by down regulating key pathways, irrespective of the cause, take advantage of the biology that is similar across disease states and may have broader clinical application. Lactoferrin has the potential to do both, by non-specifically mitigating the innate immune response as well as targeting a specific mechanism that may be driving inflammation.

Lactoferrin is an endogenous iron-binding protein (member of transferrin family) found in milk and other body fluids that has anti-microbial properties important for host defense, anti-cancer properties and immunomodulatory properties that reduce inflammation.⁴⁹ Of particular relevance is that lactoferrin suppresses production and release of pro-inflammatory cytokines (e.g., TNF- α , IL-1 β , and IL-6)⁵⁰⁻⁵² from stimulated monocytes, and enhances secretion of anti-inflammatory cytokines (e.g., IL-10, IL11, and IL-4) from intestinal myofibroblasts.^{53, 54} In studies of post-menopausal women and during pregnancy, lactoferrin treatment led to large reductions in circulating IL-6 levels (44-64%).^{55, 56} Currently, recombinant lactoferrin has been safe and well tolerated in human studies and is being studied in the general population to treatment anemia and inflammation in the elderly, to prevent sepsis in infants, in treatment of sepsis in adults, to prevent antibiotic associated diarrhea and in treatment of *Clostridium difficile* associated diarrhea (www.clinicaltrials.gov).

There are no clinical data of lactoferrin treatment among HIV positive persons, though a biologic pretense exists for why lactoferrin may be beneficial in this context. Translocation of microbial products across damaged mucosal surfaces is hypothesized to be a key driver of ongoing systemic inflammation among HIV positive patients due to LPS mediated monocyte stimulation and immune activation. Lactoferrin binds to LPS with high affinity and interferes with LPS binding of cell surface receptors (e.g., CD14) that typically initiates broad pro-inflammatory effects.⁵⁷ While reducing innate immune activation from LPS, the lactoferrin-LPS complex may have additional positive immune effects including tolerance to LPS and potentiation of response to new pathogens.⁵⁷ Lactoferrin promotes intestinal cell growth and cell migration, and decreases gut leakage caused by LPS, suggesting it may have potential to restorative mucosal integrity and the gut barrier.^{58, 59} Lactoferrin also has broad antimicrobial effects through lowering the iron concentrations and properties attributed to the positively charged N-terminal region, which is directly bactericidal and may disrupt HIV entry into cells via interactions with surface receptors (e.g., CCR5 and CXCR4).^{60, 61} Finally, lactoferrin as ribonuclease enzymatic activity, which may inhibit transcription of retroviruses by destroying the RNA genome.⁶²

In summary, lactoferrin is a safe endogenous compound, and may be uniquely beneficial for HIV positive patients due to properties that both:

- A) Mitigate endotoxin-mediated inflammation, a hypothesized mechanism driving immune activation that is specific to HIV infection, and
- B) Down-regulates innate immune activation, thus, targeting the inflammatory response more broadly, somewhat independent of the underlying mechanism.

1.2. Objectives

Our general goal is to evaluate the potential effectiveness of recombinant human (rh) lactoferrin (1500mg bid) for reducing systemic inflammation among HIV positive participants. We will also generate preliminary data on the effects on coagulation and the intestinal microbiome. We will conduct a pilot study of rh-lactoferrin using a cross-over placebo-controlled trial of 50 HIV positive participants with viral suppression receiving ART, with the following aims:

1. Evaluate the tolerability and adherence of rh-lactoferrin among ART-treated HIV positive participants at low risk for AIDS complications, to inform design of a larger clinical trial.
2. Evaluate the potential for rh-lactoferrin to reduce risk for non-AIDS-defining conditions (e.g., CVD) among virally suppressed HIV positive participants, by determining the treatment effect on an IL-6/D-dimer score that itself is associated with risk for clinical events.
3. Explore the short-term anti-inflammatory effects of rh-lactoferrin (e.g., decline in IL-6 levels and immune activation cellular phenotypes) among ART-treated HIV positive patients.
4. Explore the short-term anti-coagulant activity of rh-lactoferrin (e.g., decline in D-dimer levels and tissue factor activity) among ART-treated HIV positive patients.
5. Explore the short-term treatment effects of rh-lactoferrin on the intestinal microbiome among a subset of n=20 participants.

1.3. Trial Design

We will study rh-lactoferrin 1500mg bid in a placebo-controlled randomized, cross-over study conducted at HCMC (**Table 1**). The sequence of active study drug and matched placebo will be randomized, and a minimum two-month wash-out period will occur between the 1st and 2nd study phase. The sequence, will then proceed as follows: 1) following consent, completion of baseline visit (month '0'), and randomization, participants will begin taking study medication (rh-lactoferrin/placebo), 2) participants will follow-up at month 1 and 3, and then stop study medication, 3) after a 2 month washout period (not to exceed 4 months), they will follow-up at month 5 when they will again start the next study medication (rh-lactoferrin/placebo) in the randomized sequence, and 4) follow-up will then occur at similar intervals, now months 6 and 8. Study staff will call participants between month 1 and 3 visits, and between month 6 and 8 visits, to encourage adherence to study medication.

Table 1: Placebo-controlled cross-over trial design								
Month:	0	1	2	3	5	6	7	8
Intervention (randomized):	Phase 1				Phase 2			
Group A	Rh-lactoferrin			off	Placebo			
Group B	Placebo			off	Rh-lactoferrin			
Visits:	x	x		x	x	x		x

2. METHODOLOGY

2.1. Study Population

The target population is HIV positive patients receiving effective treatment with ART and at low risk for AIDS defining complications. For these participants, non-AIDS defining long-term complications

(e.g., CVD and cancer) dominate the spectrum of morbidity and mortality. We will also focus on participants over age 40, as age also increases risk for many non-AIDS defining clinical complications. Any antiretroviral medications can be used. No restrictions are present on concurrent medications, but participants with common diseases known to be pro-inflammatory will be excluded.

Participants will be recruited from the HCMC HIV clinic (PCC; Positive Care Center) through provider referral and informational flyers distributed in clinical exam rooms. The PCC provides care for over 1700 HIV positive participants, and approximately 75% are receiving ART with suppressed viral load and over age 40 (i.e., potentially eligible). Referrals will also be accepted from local providers in the Twin Cities. We anticipate that recruitment and enrollment last approximately 1 year. Recruitment progress will be assessed weekly with research staff.

2.2. Eligibility Criteria

Inclusion Criteria:

- 1) HIV-positive participants receiving ART for >1 year
- 2) HIV RNA level <200 copies/mL for >1 year (≥2 separate values)
- 3) Age ≥40 years

Exclusion Criteria:

- 1) Pregnancy
- 2) Rheumatology diseases
- 3) Treatment with immune-modulatory drugs
- 4) Treatment with hepatitis C therapy
- 5) Treatment with anti-coagulant medication (not including aspirin)
- 6) Invasive cancer in the prior year or receiving cancer treatment (carcinoma-in-situ and basal cell cancer of the skin are not consider invasive cancer)
- 7) Chronic kidney disease, stage IV or V (creatinine clearance <30 mL/min/1.73m²)
- 8) Cirrhosis or end-stage liver disease
- 9) Gastro-intestinal infection within the prior month
- 10) Assessment by the clinical investigator that enrollment into the study could entail excess risk to the participant, beyond what is intended or expected.

2.3. Intervention

All 50 participants will take rh-lactoferrin 1500mg bid (twice daily) and matched placebo, sequentially in a cross-over design with the order randomized (see design **table 1** above). Rh-lactoferrin, and matched placebo, study drug in capsule form will be obtained through Ventria Bioscience (see investigator brochure, **appendix C**). Study medications will be stored at -20F in a secure location and dispensed by study nurses, consistent with HCMC pharmacy policies on use of study medications for IRB-approved clinical research.

2.4. Outcomes

Primary Outcomes

- a. Study medication tolerability

- i. Study withdrawal or study drug discontinuation due to medication side effect (subjective or toxicity laboratory criteria)
 - ii. Toxicity lab measure with >2 fold increase (e.g., BMP, LFTs, CBC)
- b. Study medication adherence
 - i. Self report of missed doses
 - ii. Objective assessment – estimated via the proportion of pills taken by pill count
- c. Changes in IL-6 and/or D-dimer over 3 months (change while on rh-lactoferrin compared to change while on placebo), including changes in a composite score that considers changes in both IL-6 and/or D-dimer levels (see analysis plan)

Secondary Outcomes

The potential treatment effect of rh-lactoferrin on the biologic pathways and end-organ systems listed below will be assessed as secondary outcomes. The precise number of markers tested for each pathway will depend on primary outcome biomarker findings, and on availability of funding to support the additional laboratory work.

- a. Rh-lactoferrin drug levels in serum at steady state
- b. Changes in markers of innate immune activation (e.g., monocyte phenotypes, sCD14, sCD163, TNF- α).
- c. Changes in the prevalence of CD4+ and CD8+ T-cell phenotypes (e.g., HLA-DR+/CD38+)
- d. Changes in coagulation activation (e.g., tissue factor functional activity, tissue factor expression on monocytes, thrombin-antithrombin complexes)
- e. Changes in ferritin and clinical labs of iron binding (e.g., TIBC)
- f. Changes in markers of microbial translocation (e.g., lipopolysaccharide levels [LPS], 16S rDNA, zonulin, I-FABP [intracellular fatty acid binding protein])
- g. Changes in HIV persistence (e.g., via estimates of HIV complement DNA levels or single copy RNA levels in peripheral blood)
- h. Changes in small artery elasticity (SAE), a measure of microvascular dysfunction
- i. Changes in markers of bone health (e.g., RANK ligand, osteocalcin)
- j. Associations between neurocognition and biomarkers and immune activation phenotypes

2.5. Time-to-Events for Study Visit Procedures

If eligibility criteria are met, participants will be randomized at the baseline visit to start active study drug or matched placebo (blinded). Randomization will occur within 2 months of screening. After enrollment and baseline visits, participants will present months 1, 3, 5, 6 and 8 for repeat study procedures. The 1st drug will be stopped at the month 3 visit, and the 2nd study drug will be started at the month 5 visit. The visit schedule is outlined in the table below. Study visits will last approximately 60 minutes. Patients will be fasting for all blood draws (except screening).

Toxicity labs and a clinical assessment is included as part of the follow-up study visits. If new symptoms develop that may be related to study medications, the study investigators will be notified within 24 hours and additional toxicity labs and adherence will be assessed.

Table 2: Study procedures and visit timeline

Measure	Screening visit	Baseline visit	As Needed Symptom Assessment	Follow-up Study Visits
Informed consent	X			
Randomization		X		
HIV RNA level (≤3 months)	X (must be ≤3 months from screening)	X (if not done at screening)	X	X
CD4 count		X	X	X
Hep B/C serologies (≤12 mo.)		X		
Lipid panel (≤12 months)		X		
Toxicity labs (BMP, LFTs, CBC) (≤3 months)	X (must be ≤3 months from screening)	X (if not done at screening)	X	X
Toxicity labs (CK, ferritin, serum iron, TIBC, transferrin)		X	X	X
Plasma/serum/PBMCs		X		X
Urine pregnancy test (women)	X			
Medication Adherence			X	X
Artery Elasticity		X		X
Stored Plasma/Blood/PBMC		X		X
MoCA		X		

2.6. Sample Size Considerations

Medication and tolerability will be assessed though were not considered for sample size calculations.

Power estimates were calculated for a composite IL-6/D-dimer score that adjusts for regression dilution bias. With 80% power and $\alpha=0.05$, we can detect a 0.40 standard deviation difference (rh-lactoferrin compared to placebo) in the composite score. That difference would be found with 10% decline in IL-6 and a 5% decline in D-dimer, a 7% decline in IL-6 and a 10% decline in D-dimer, or a 12% decline in IL-6 and no change in D-dimer. The corresponding predicted relative risk reduction with a 0.40 standard deviation effect size is approximately 13% for serious non-AIDS or death, and 10% for CVD events (acute MI, stroke, revascularization, or CVD death).

2.7. Randomization and Implementation of Treatment Sequence

The sequence of study drug administration (i.e., active rh-lactoferrin and matched placebo) will be randomized in this cross-over design. Randomization sequence will be predetermined for a set of unique study drug bottle identification numbers using block-randomization with varied groups of 4 and 8, and blinded to investigators, staff and participants. An un-blinded statistician, one research staff not involved with study visits, and an HCMC pharmacist will have access to the randomization code linked to study drug bottle numbers. Once a study participant is enrolled and randomized they will be assigned a study drug bottle number, which will be dispensed per protocol.

3. CLINICAL MANAGEMENT

3.1. Study Visit Procedures

Study nurses at the HCMC Positive Care Center (PCC) Research Program will be trained prior to study implementation and will perform all study visit procedures during the conduct of the study. Study visits will take approximately 60 minutes.

3.1.1. Data Collection

All data will be entered into web-based case report forms and transferred to servers at the data coordinating center at University of Minnesota School of Public Health using REDCap software. Paper source documentation will also be kept in a secure, locked location in the PCC research program. Study nurses will perform all blood draws, and a lab technician will assist with blood specimen processing immediately following blood draw. The blood specimen collection tubes and processing protocols are outlined in the PCC research laboratory manual.

3.1.2. Adherence and Tolerability

Adherence will be assessed via participant self-report and pill count (by study nurse) during each follow-up visit. Tolerability will also be assessed via self-report, with additional laboratory assessments performed for toxicity as listed below.

3.1.3. Blood Specimen Processing

See PCC research laboratory manual for details on blood specimen processing. Participants will be fasting for all study visit blood draws (optional for screening visit). Plasma, serum and peripheral blood mononuclear cell (PBMC) specimens will need to be processed from whole blood within 30 minutes of collection. Plasma is isolated after centrifuge at 4°C at 2500 X g for 15 minutes (for a total of >30,000 g-minute spin); this process is repeated TWICE to obtain platelet-poor plasma. Whole blood is placed directly in cryovials prior to centrifugation. PBMC processing will occur at the University of Minnesota within 2 hours of blood draw.

3.1.4. Clinical Labs

All clinical labs will be measured on fresh blood specimens the day of the study visit, at the HCMC clinical lab using CLIA approved standards. Plasma HIV RNA level will be estimated using COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0.

The following clinical labs will be assessed at each visit to monitor for toxicity:

- i. Complete Blood Count (CBC, with platelet count and differential)
- ii. Complete Metabolic Panel (CMP, includes hepatic panel)
- iii. Creatine Kinase (CK)
- iv. Iron Studies (ferritin, serum iron, total iron binding capacity, transferrin saturation)

3.1.5. Rectal Swab Specimen Collection

Intestinal microbiome substudy (n=20) will entail collection of a rectal swab specimen before and after study drug for both phase 1 and phase 1 (see table 1), corresponding to 4 visits at baseline and month 3, 5 and 8). Specimens are collected via a sterile cotton tip applicator, placed into a cryovial, frozen in liquid nitrogen, and stored at -80F until analysis. Microbiome sequencing studies will then be coordinated by Dr. Sereti at NIAID/NIH. Briefly, DNA will be extracted from rectal swab specimens, and the intestinal microbial community will be profiled via 16S rRNA gene amplicon sequencing. The relative frequencies of different taxa will then be computed for analyses.

3.1.6. Blood Pressure Waveform Analysis

Blood pressure waveform data will be collected consistent with previously established protocols implemented at HCMC and for multi-center studies (e.g., NHLBI-funded substudy of Strategic Timing of AntiRetroviral Therapy trial, and the Multi-Ethnic Study of Atherosclerosis).⁸⁰⁻⁸³ Briefly, analysis of the diastolic blood pressure waveform will be performed using a modified windkessel model of the circulation (HDI/PulseWave CR-2000, Eagan, MN) to calculate large and small artery elasticity (ml/mmHg; LAE and SAE respectively). A typical waveform will have two maxima in the diastolic curve. The first represents capacitance of the proximal aorta and major branches following cardiac ejection (LAE). A second oscillatory reflective wave represents elasticity in small arteries (SAE). During the visit, a 30-second measure of the radial pulse waveform is achieved in triplicate.

3.1.7. Neurocognitive Assessment

Utilizing the Montreal Cognitive Assessment (MoCA), we will explore the relationships between biomarkers of inflammation (and cellular activation phenotypes) and cognition. The MoCA has been validated in various chronic illness states, including HIV (<http://www.mocatest.org/references.asp>).⁸⁴ The MoCA is a pen and paper, 12-item questionnaire that takes approximately 10 minutes to complete. The MoCA questionnaire is included in appendix B.

3.2. Laboratory Outcomes

3.2.1. Plasma Biomarkers

Plasma specimens will be analyzed at the Laboratory for Clinical Biochemistry Research (U of Vermont), under the direction of Dr. Russell Tracy. IL-6 will be measured with Chemiluminescent Sandwich ELISA (R&D Systems), and D-dimer levels with immunoturbidometric methods on the Sta-R analyzer, Liatest D-DI (Diagnostica Stago). A high degree of repeatability (low analytic variability) has been reported by Dr. Tracy's lab;⁶⁵⁻⁷¹ the coefficient of variance was 7% for IL-6, and 12% for D-dimer.^{66, 67, 72} Additional inflammatory biomarker levels will be measured with ELISA or multiplex assay kits (MesoScale).

3.2.2. Immunologic Phenotypes

Immunophenotyping will be performed on cryopreserved PBMCs using multi-color flow cytometry at an intramural NIAID/NIH laboratory under the direction of Dr. Irini Sereti. Panels of fluorochrome-conjugated antibodies for cell surface markers not affected by cryopreservation (e.g., CD3, CD4,

CD8, CD38, HLA-DR, CD14, CD16, CD142 or tissue factor (TF) and viability dye to exclude non-viable cells) have been validated previously by Dr. Sereti.⁷³ The prevalence of classical (CD14+/CD16-), intermediate (CD14+/CD16+), and non-classical (CD14^{dim}/CD16+) monocyte phenotypes and activated T cells phenotypes (HLA-DR+/ CD38+) will be characterized, and with these methods additional phenotypes may be easily characterized (e.g. TF expression on monocytes, T-regulatory cells, or senescent T-cells). Only live cells will be included; samples with low (<75%) viability will not be processed.

3.2.3. Tissue Factor Activity

Tissue Factor (TF) activity will be assessed at Dr. Nigel Key's laboratory at the University of North Carolina. MP-TF pro-coagulant activity will be measured using a chromogenic assay whereby the TF-dependent factor Xa generation is determined by subtracting the amount of FXa generated in the presence of anti-TF1 human antibody from that generated in the presence of a control antibody.⁷⁴ TF-positive microparticles (MP-TF) are highly procoagulant, reflect cell-free TF, and have been linked to thrombosis in a variety of diseases.⁷⁵⁻⁷⁸ Whole blood tissue factor (TF) pro-coagulant activity will assess the full contribution of circulating TF, including both cell-associated and cell-free (e.g., MP-bound). Whole blood is processed without separating cellular elements from plasma, and frozen at -70 C. Freeze/thaw cycles are then applied to lyse cells, and lysed blood is centrifuged to isolate a membrane pellet, which is suspended in bovine serum albumen. TF activity is then assayed using a two-stage functional clotting assay.⁷⁹

4. SAFETY AND ADVERSE EVENTS

4.1. Adverse Event Definitions and Collection

4.1.1. Adverse Event (AE): Adverse event (AE): Any untoward medical occurrence in a clinical research participant administered an investigational product and which may or may not have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product.

If a preexisting condition worsens post-enrollment (frequency increases and/or severity grade increases), it should be reported as an adverse event.

Appendix D provides a table of adverse events, with grading criteria, adopted from the DAIDS 2009 Table for Grading the Severity of Adult and Pediatric Adverse Events

4.1.2. Serious Adverse Event (SAE): Any adverse event occurring at any dose that results in any of the following outcomes:

- i. Death,
- ii. A life-threatening condition,
- iii. A congenital anomaly/birth defect,
- iv. Inpatient hospitalization or prolongation of existing hospitalization,

- v. Persistent or significant disability/incapacity,
- vi. An important medical event that, based upon appropriate medical judgment, may jeopardize the patient or subject and may require intervention to prevent one of the outcomes listed above.

4.1.3. Symptom Check List: In addition to an open-ended question at each study visit, specific symptoms will be screened for adverse events related to: headache, nausea, vomiting, diarrhea, abdominal pain, rash. Laboratory data to monitor for potential toxicity will include: serum creatinine, hemoglobin, white blood count, platelets, aspartate aminotransferase, alanine aminotransferase, and total bilirubin.

4.2. Grading and Documenting Adverse Events

All adverse events during the study period will be ascertained and recorded on study visit CRFs (through the online RedCAPS interface). Serious adverse events will also be described via a narrative form (appendix E) to be included in source documentation.

Each adverse event will receive a grade based on severity, and will be assigned a 4-digit numeric 'event code'. Criteria for assigning a grade of severity of adverse events, and the corresponding event code, are summarized in Appendix D.

There are five severity grades that can be assigned to adverse events, which are defined as follows:

- i. Grade 1 = Mild
- ii. Grade 2 = Moderate
- iii. Grade 3 = Severe
- iv. Grade 4 = Potentially life-threatening
- v. Grade 5 = Death

NOTE: information on adverse event do NOT need to be recorded if < grade 2, and adverse events related to CD4+ T-cell count criteria will NOT be ascertained or recorded.

Additional information ascertained for each adverse event includes: a) date of onset, b) relationship to study drug (see section 4.3 below), c) any action taken on study drug, d) the outcome of the adverse event (e.g., resolved or ongoing), and e) whether the adverse event qualifies as a serious adverse event (including the SAE criteria fulfilled).

The narrative to be included in source documentation (appendix E, in addition to the CRF variables for AEs) will be required for all serious adverse events, as well as any other problems involving risks to participants that is unanticipated or unexpected and is reasonably believed to be related to research participation. The circumstances surrounding these events should be recorded using the serious adverse event CRF (Appendix E)

4.3. Adverse Event Relationship to Study Drug

For all adverse events that occur after baseline that are ascertained and recorded in a study CRF, study investigator(s) will assess the potential relationship of the event to the study medication. One of the following designations will be used:

- i. Probably Related: the adverse event and administration study drug are reasonably related in time, and the adverse event is more likely explained by rh-lactoferrin than other causes.
- ii. Possibly Related. The adverse event and administration study drug are reasonably related in time, and the adverse event could be explained equally well by causes other than rh-lactoferrin.
- iii. Not Related. The adverse event is clearly explained by an alternative explanation or known association with another medication(s) or medical condition, not related to rh-lactoferrin.

4.4. Reporting Serious Adverse Events

Instructions on criteria for submitting safety reports to the FDA and the process for submitting safety reports, in accordance with IND requirements, can be found at: [Guidance for Investigators on Safety Reporting Requirements](#) (Sections V and VII, respectively)

The HCMC IRB (or Human Subjects Committee), the FDA, and Ventria Bioscience will receive concurrent safety reports for all adverse events that are **BOTH serious and unexpected**, and related to study medication. Time frame for reporting will be:

- i. FDA: Reports will be submitted within **15 calendar days** after the sponsor determines that the suspected adverse event or other information qualifies for reporting. Unexpected life-threatening or fatal suspected adverse events will be reported within **7 calendar days** of the sponsor's receipt of the information.
- ii. HCMC IRB: Reports for serious adverse events related, or possibly related, to study drug will be sent within **5 working days** of knowledge of the event. Serious adverse events not related to study medication will be reported within **30 days** of knowledge of the event.
- iii. Ventria: Will be notified via email within 72 hours of the sponsor knowing of the serious adverse event, as reports are being prepared. Reports sent to FDA or HCMC IRB will be sent to concurrently to Ventria

Safety reports for any serious adverse events submitted to IRB, FDA and Ventria, will utilize the same format. The following documentation will then be completed:

- i. fill out an adverse event case report form, via REDCap
- ii. fill out an FDA MedWatch form 3500A
- iii. complete FDA cover letter and FDA form 1571
- iv. email the completed cover letter, FDA form 1571, and FDA MedWatch form 3500A and to:
 - 1. Study PI, Jason Baker: baker@umn.edu
 - 2. DCC, Katherine Huppler Hullsiek: kathy-h@ccbr.umn.edu
 - 3. FDA/IND, Katherine Schumann: Katherine.Schumann@fda.hhs.gov
 - 4. Ventria Bioscience Contacts:
 - a. Ning Huang: nhuang@ventria.com
 - b. Seymour Fein: sfein@ventria.com
 - c. Reid Snowden: rsnowden@ventria.com
- v. Notify the HCMC/MMRF IRB, by emailing FDA MedWatch form 3500A and a new cover letter

Aggregate bi-annual reports will also be created that include information on unexpected adverse events or any unanticipated problem that poses risks to participants, and is believed to be related to

research participation. These bi-annual reports will be submitted to HCMC IRB and Ventria. Annual aggregate reports on all serious and unexpected adverse events will be submitted to the FDA and Ventria, per respective reporting requirement.

Link to: [All FDA MedWatch reporting forms \(including Form 3500A\)](#)

Link to: [instructions for completing FDA form 3500A](#)

Link to: [FDA form 1571](#) and [instructions for completing FDA form 1571](#)

Link to: [FDA MedWatch voluntary reporting online](#)

4.5. Study Drug Modification or Participant Withdrawal

At each study visit, participants will undergo clinical and laboratory assessments to assess for adverse events or any potential side effect to study medication. Clinical labs to assess toxicity are listed in section 3.1 above (clinical assessment), and these may be repeated at any point during the study: a) if participants experiences subjective symptoms, signs or laboratory results suggestive of drug toxicity/intolerance, or b) to follow-up on laboratory abnormalities of grade ≥ 3 , or c) at the discretion of the study investigator(s) to assess for potential new or worsening study drug toxicity.

Participants may withdraw from the study at any time at their request, as described in the consent.

Participants will be un-blinded to study medication, and will stop any active drug, **for any adverse event of grade ≥ 3 that are deemed probably or possibly related to study medication**. For other adverse events, study investigator(s) will evaluate if participant safety necessitates a modification to study drug with options including, but not limited to, temporarily holding study medications and re-assessing (e.g., for a period of ~1-2 weeks). Given the short-term follow-up time frame on study medication, if periods off study medication are anticipated to be longer than several weeks participants may be asked to withdraw from the study or stop study medication.

All randomized participants will be encouraged to complete a final study visit, including all visit procedures, upon withdrawal.

4.6. Data and Safety Monitoring Plan

To ensure that study data are reported correctly and completely, data will be on-site monitored at least annually for IRB approval, compliance with all applicable regulatory guidelines, participant eligibility and retention, and the reporting of all adverse events. The monitor will be independent of the clinical site and must have the appropriate scientific and clinical knowledge to monitor the study.

Participant safety data will be summarized and presented by the study statistician to an independent committee composed of at least one clinician and at least one biostatistician. This review will occur at the earliest of six months after the first randomization or when 25 participants have been randomized to the study, and again on annual basis from the time of the first randomization. Committee members

will have experience with HIV/AIDS clinical research studies. Data presented will include recruitment, tolerability (including medication side effects, adherence and discontinuations), adverse events and toxicity labs.

5. ANALYSIS PLAN

The general goal of this pilot study is to gather data on tolerability, adherence, feasibility and potential efficacy for lowering inflammation and coagulation activation. Utilizing a placebo-controlled cross-over design (n=50 participants), we hypothesize that rh-lactoferrin given at 1500mg twice daily will be tolerable, be associated with high adherence, and will lower plasma IL-6 and D-dimer levels.

We will calculate the change in a composite score that considers changes in both IL-6 and/or D-dimer (IL-6/D-dimer score, see Rationale and Sample Size Considerations above) using a variety of metrics, including averaging follow-up values and using longitudinal mixed models to account for the within person correlation. We will also estimate the magnitude of the posited risk reduction in serious non-AIDS events (e.g., CVD and cancer) or all-cause mortality corresponding to the treatment effect of recombinant rh-lactoferrin.

Comparisons entail treatment versus placebo as randomized, with participants serving as their own comparison. Possible interactions with sequence or period effects will be assessed. Data will be transformed and analyses adjusted for covariates as necessary. Secondary outcomes will be analyzed similar to primary comparisons.

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APPENDIX A1: INFORMED CONSENT TEMPLATE (MAIN STUDY)

HSR #13-3657

7-August-2014

Recombinant Lactoferrin to Reduce Immune Activation and Coagulation Among HIV Positive Patients

Funded by Hennepin Health Services

Short Title of the Study: rh-Lactoferrin HIV Study

CONSENT FOR PARTICIPATING IN A RESEARCH TRIAL

INVESTIGATORS: Jason Baker, MD, MS, Rachel Prosser, PhD, RN, Keith Henry, MD
PHONE: 612-873-2705

INTRODUCTION AND PURPOSE: WHY IS THIS STUDY BEING DONE?

Heart disease, or cardiovascular disease, is now a leading cause of illness and premature death among persons with HIV-infection. Safe, effective, and inexpensive treatment strategies that reduce risk for heart disease specifically for persons with HIV infection are needed.

The purpose of this study is to test the feasibility and tolerability of giving human recombinant (rh) lactoferrin to patients with HIV infection. Lactoferrin is an iron-binding protein found in milk and other body fluids that has anti-microbial properties important for host defense, and immunologic benefits that reduce inflammation. Findings from this study will support additional research to determine if this naturally occurring protein could be effective in reducing cardiovascular event risk in persons who are HIV positive, by decreasing inflammation and coagulation ('blood clotting') abnormalities.

Human recombinant (rh) lactoferrin is identical to the human lactoferrin protein, but is it produced by rice after inserting the lactoferrin gene into the rice genome. Rh-lactoferrin is not currently approved by the FDA (Federal Drug Administration) for clinical use, but is being studied as treatment for several diseases and no safety concerns have been identified.

YOUR PARTICIPATION IS VOLUNTARY

This consent form gives you information about the clinical research study that will be discussed with you. Once you understand the study, and if you agree to take part, you will be asked to sign this consent form. You will be given a copy to keep.

Before you learn about the study, it is important that you know the following:

- Your participation is entirely voluntary;
- You may decide not to take part or to withdraw from the study at any time without losing the benefits of your routine medical care.

Eligibility: Who is being asked to be part of this research study?

APPENDIX A1: INFORMED CONSENT TEMPLATE (MAIN STUDY)

HSR #13-3657

7-August-2014

You are eligible for this study because you are over age 40, you have HIV infection and you are doing well with taking your antiretroviral medications. Specifically your HIV 'viral load' must be undetectable. If your doctor or the study investigators feel that it would not be safe to take the study medication then you will not be eligible to participate.

HOW LONG WILL YOU BE IN THE STUDY?

If you qualify for this study and sign this consent after your screening visit, you will be enrolled in the study. The study will then last approximately 8 months including the screening visit. After screening, you will come in for a 'baseline' study visit, and then a follow-up visit at 1, 3, 5, 6 and 8 months.

HOW WILL THE STUDY WORK?

If you agree to participate in this study, you will be asked to take 1500mg of rh-lactoferrin twice daily and matching placebo, one after the other (i.e., not at the same time). At the beginning of the study you will be randomly assigned to start one study medication, which will either be rh-lactoferrin or placebo. At month 3 you stop taking study medications. At month 5 you will be asked to start the second study medication, which will be placebo if your first medication was rh-lactoferrin but will be rh-lactoferrin if your first medication was placebo. Neither you, nor the study investigators, will know which study medication you take first or second.

PROCEDURES: WHAT DO YOU HAVE TO DO IF YOU ARE IN THIS STUDY?

Screening visit:

You will be asked to come in for a screening visit, where your study investigators will review the study procedures and this consent form. If you agree to participate, you will have your blood drawn (approximately 2 tablespoons) and medical history reviewed (similar to a clinic visit) to determine if you are eligible to participate. If you meet study criteria, you will then return within 1 month to begin the study.

Baseline and Follow-up visits:

Each visit will last approximately one hour, and will consist of:

- 1) Medical History: You will be interviewed to review your medical history and assess your risk for developing heart disease. We will also access your medical chart to obtain the results of recent lab tests and medications you are taking.
- 2) Blood Draw: We will obtain a blood sample (approximately 8 tablespoons) from a vein in your arm. The total blood draw volumes per visit will be up to 100 – 110 mL. You must be fasting for at least 6 hours prior to having your blood drawn at study visits. Blood samples will be used to measure markers in the blood related to inflammation and other markers that may contribute to heart disease and other complications of HIV infection. These samples may be stored for up to 20 years.

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- 3) **Blood Pressure Waveform Analysis:** We will record your pulse waveform measured at your wrist for a brief period while you are resting (using a portable device: HDI/CR-2000). This involves no more discomfort than getting your blood pressure checked. This device estimates how well your vessels are functioning, including the ability of your vessels to relax. Your blood pressure, height and weight will also be measured.
- 4) At follow-up visits you will be asked to bring in all of your study medication so that adherence and tolerability to the treatment can be assessed.
- 5) At all study visits you will be asked to complete a questionnaire that assesses your memory, language, attention and abstract thought, called the Montreal Cognitive Assessment (MoCA). The MoCA is a 1-page pen and paper 12-item questionnaire. This should take you approximately 10 minutes to complete.

HOW WILL YOU GET MEDICINES FOR THE STUDY?

Study medications will be provided to you by the study and will be distributed by study nurses during study visits. You will be provided with sufficient supply to last the duration of the study. You will be asked to return any unused study medication, including empty containers, at the final study visit.

WHAT ARE THE RISKS AND/OR DISCOMFORTS OF THIS STUDY?

Possible risks

You will be monitored for side effects at each visit, and your lab tests will include an evaluation for signs of medication toxicity. There are no known side effects due to rh-lactoferrin given at the proposed amount, but it has not been studied in HIV patients and you will still be monitored for tolerability, any toxicity, and any changes in the efficacy of your HIV treatment specifically.

Risks of Study Procedures

There are no known risks associated with having your pulse waveform measured. You will have your blood drawn at each study visit. This is identical to having your blood drawn at a medical clinic, and can involve discomfort and/or minor bruising.

WHAT ABOUT PREGNANCY AND BREASTFEEDING?

If you are pregnant, breastfeeding or planning to become pregnant, you will not be eligible for this study. If you become pregnant during the study, you will be asked to withdraw. Therefore, if it is possible that you could become pregnant, we ask that you use at least 1 form of birth control (condoms, etc.) or abstain from sex during the study period.

WHAT ARE THE BENEFITS OF THIS STUDY?

If you take part in this study, there are no anticipated long-term benefits to your health. The treatment may improve inflammation, but the short duration is unlikely to affect your health. What we learn from this study may help us prevent heart disease and improve the treatments of other people who are infected with HIV.

APPENDIX A1: INFORMED CONSENT TEMPLATE (MAIN STUDY)

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COMPENSATION

You will be paid \$20 for each study visit you attend after the screening visit. You may receive a total of \$160.00 for participating in the study. Study investigators may ask for your social security number as part of the monitoring process for this compensation.

WHAT IF THERE ARE NEW FINDINGS?

We will not be analyzing data during the study. However, if during the course of this research study, there are significant new findings discovered which might influence your willingness to continue, the researchers will inform you of those developments. You may request your own results after the study by contacting the research investigators.

WHAT IF YOU DON'T WANT TO BE IN THE STUDY ANY LONGER?

If you enroll in this study, you may decide to stop participating at any time. Withdrawing from this study will not affect the benefits of your regular medical care.

CAN YOUR STUDY PARTICIPATION BE STOPPED WITHOUT YOUR CONSENT?

You may be taken off of study medicines before the end of the study if study investigators or your doctor recommend this. You may be taken off the entire study without your consent if:

- Your study doctor decides that continuing in the study would harm you;
- Your lab results indicate that you are experiencing toxicity from study medications;

WHAT OTHER CHOICES DO YOU HAVE BESIDES THIS STUDY?

You may discuss other strategies for reducing your risk for heart disease with your doctor. You will also be given general information about ways to decrease your risk for heart disease at the beginning of this study. Rh-lactoferrin is not available for clinical use.

WHAT ARE THE COSTS TO YOU?

The medications that are part of this study will be provided free-of-cost to you, and will be distributed during study visits. During the study, you, your insurance company, or some other third-party payer must pay for all other medicines, including HIV medicines not paid by the study and medicines needed to prevent or treat other illnesses. We will provide all clinical and professional services, lab work, and other tests that are part of this study and not part of your regular care at no cost to you.

HOW IS YOUR PRIVACY PROTECTED?

Any information that could be used to identify you will be treated in strict confidence to the extent allowed by law. Nevertheless, some uses and disclosures of your information are necessary to conduct the study. If you agree to be part of this study, you will also be allowing the uses and disclosures of your private health information as needed for the purposes of this study as described in this consent.

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“Private health information” means information that identifies you and is collected:

- during this study;
- from your past and current medical records maintained by your regular health care providers (including, if applicable, HCMC), to the extent the information is relevant to this study or to your eligibility for this study; or
- from any payment records relating to items or services furnished to you during this study.

By signing this consent, you are agreeing that your private health information may be disclosed to and used by:

- the doctors and other health care providers involved in this study;
- their staff;
- the research center (Minneapolis Medical Research Foundation);
- members of the HCMC Human Subjects Research Committee/Institutional Review Board;
- the sponsor of this study and its agents; and
- monitors from the United States Government and/or Food and Drug Administration (FDA).
- Your clinical research labs may be entered into the electronic medical record (EPIC) at Hennepin County Medical Center. These lab results may be viewed by other providers and allied health professionals.

The findings of this study may be used for scientific meetings, written reports, and publications, but no information that could be used to identify you will be disclosed for these purposes.

Once your private health information has been disclosed to a third party, federal privacy laws may no longer protect it from re-disclosure. However, anyone obtaining access to your private health information under this consent must agree to protect your information as required by this consent.

This consent to use your private health information as described above does not expire. However, if you later change your mind, you can revoke this consent by writing to Dr. Jason Baker saying that you no longer wish to allow your private health information to be used for this study. If you revoke your consent, you may no longer be able to participate in the study. Moreover, we cannot undo uses or disclosures of your private health information that have already taken place in reliance on your

As part of our ongoing research studies to better understand disease and improve health we often contact patients about clinical studies for which they may be eligible to participant. By signing this consent, you would also authorize us to contact you about future studies. If you would prefer we not contact you directly about future studies, please indicate this by placing a check after 'do not contact me' and then initialing.

Do not contact me: _____ Initial: _____

APPENDIX A1: INFORMED CONSENT TEMPLATE (MAIN STUDY)

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PLEASE NOTE:

In the event of a positive result for Hepatitis B or C, reporting of the results to the Minnesota the Department of Health is required.

WHAT IF YOU ARE INJURED?

If you are injured as a result of being in this study, you will be given immediate treatment. The cost for treatment will be charged to you or your insurance company. There is no program through this institution to compensate participants who have research related injuries. You will not be giving up any of your legal rights by signing this consent.

WHAT IF YOU HAVE PROBLEMS OR QUESTIONS?

If you ever have questions or in the case of research-related injuries, you should contact:

Jason Baker, MD, MD

701 Park Ave South; Mail Code G5

Minneapolis, MN 55415

612-873-2705

OR

Rachel A. Prosser, PhD, RN, CNP

701 Park Ave South; Mail Code O1.326

Minneapolis, MN 55415

612-873-2877

If you have questions about research subject's rights you can contact:

Dr Fred Langendorf, Chairman, Human Subjects Research Committee

701 Park Ave., S9.115

Minneapolis, MN 55415

Phone: 612-873-6882

RESEARCH STUDY REGISTRY

A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as required by U.S. Law. This Website will not include information that can identify you. At most, the Website will include a summary of the results. You can search this Website at anytime.

APPENDIX A1: INFORMED CONSENT TEMPLATE (MAIN STUDY)

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SIGNATURE PAGE FOR CONSENT TO PARTICIPATE IN THE RH-LACTOFERRIN HIV STUDY

I have read this consent form, had the opportunity to ask questions and have received answers to any questions I have asked. I willingly give my consent to participate in this study, and authorize the use and disclosure of my health information as described in this form. By signing this consent form I do not give up any of my legal rights. Upon signing this form I will be given a signed copy of the form for my records.

If you have read the informed consent (or if you have had it explained to you) and understand the information, and you voluntarily agree to join this study, please sign your name below.

_____ Participant's name (typed or printed)	_____ Participant's signature	_____ Date
_____ Signature of person obtaining consent		_____ Date

APPENDIX A2: INFORMED CONSENT TEMPLATE (MICROBIOME SUBSTUDY)

HSR #13-3657

25-March-2015

Recombinant Lactoferrin to Reduce Immune Activation and Coagulation Among HIV Positive Patients: *Microbiome Substudy*

Funded by Hennepin Health Services

Short Title of the Study: Microbiome Substudy of rh-Lactoferrin HIV Study

CONSENT FOR PARTICIPATING IN A RESEARCH TRIAL

**INVESTIGATORS: Jason Baker, MD, MS, Rachel Prosser, PhD, RN, Keith Henry, MD
PHONE: 612-873-2705**

INTRODUCTION AND PURPOSE: WHY IS THIS RESEARCH SUBSTUDY BEING DONE?

You have already agreed to take part in the main study, “rh-Lactoferrin HIV.” Safe, effective, and inexpensive treatment strategies are needed for HIV positive persons that reduce risk of diseases that are a result of ongoing ‘inflammation’ in the body over many years (such as heart disease). The purpose of the main study is to test the feasibility and tolerability of giving human recombinant (rh) lactoferrin to patients with HIV infection.

A focus of the main study is to see if rh-lactoferrin reduces inflammation by improving immune function in intestinal tissues (‘the gut’) of people with HIV infection. One consequence of HIV damage to the gut is an alteration in the composition, diversity and function of gut bacteria—this is sometimes called ‘dysbiosis.’ This dysbiosis is thought to contribute to ‘inflammation’ in the gut and throughout the body. The purpose of this ‘microbiome substudy’ is to determine if rh-lactoferrin helps normalize the gut bacteria, or improve dysbiosis.

This substudy will include approximately 20 participants that have co-enrolled into the main rh-Lactoferrin HIV study. Findings from this study will support additional research to determine if this naturally occurring protein could be effective in reducing heart disease or other clinical events among persons who are HIV positive, by decreasing inflammation and intestinal health.

Human recombinant (rh) lactoferrin is identical to the human lactoferrin protein, but is it produced by rice after inserting the lactoferrin gene into the rice genome. Rh-lactoferrin is not currently approved by the FDA (Federal Drug Administration) for clinical use, but is being studied as treatment for several diseases and no safety concerns have been identified.

YOUR PARTICIPATION IS VOLUNTARY

This consent form gives you information about the clinical research substudy that will be discussed with you. Once you understand the study, and if you agree to take part, you will be asked to sign this consent form. You will be given a copy to keep.

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Before you learn about the substudy, it is important that you know the following:

- Your participation is entirely voluntary;
- You may decide not to take part or to withdraw from the substudy at any time without losing the benefits of your routine medical care.

Eligibility: Who is being asked to be part of this research substudy?

You are eligible for this substudy because you are participating in the main study. If your doctor or the study investigators feel that it would not be safe to participate in this substudy, or the main study, then you will not be eligible to participate.

HOW LONG WILL YOU BE IN THE STUDY?

If you qualify for this substudy and sign this consent, you will be enrolled in the substudy. Taking part in this sub study will not require any additional visits. The substudy procedures will occur at the same time as one of your regularly study visits for the main study.

HOW WILL THE STUDY WORK?

If you agree to participate in this microbiome substudy, you will have one extra procedure performed at the following study visits: Baseline, Month 3, Month 5 and Month 8.

PROCEDURES: WHAT DO YOU HAVE TO DO IF YOU ARE IN THIS SUBSTUDY?

Rectal Swab

You will lie on your side on an exam table. The study doctor or a research staff member will briefly insert a sterile cotton swab (like a Q-tip) through the anus into the rectum. The swab will be inserted 1-2 inches and the procedure will last 5-10 seconds.

WHAT ARE THE RISKS AND/OR DISCOMFORTS OF THIS SUBSTUDY?

Risks Of Rectal Swab

You may feel pressure as a swab is inserted into the rectum, but the test is usually not painful. Having the swab performed may also cause embarrassment or make some participants emotionally uncomfortable. The procedure is very brief and will last only seconds.

WHAT ABOUT PREGNANCY AND BREASTFEEDING?

If you are pregnant, breastfeeding or planning to become pregnant, you will not be eligible for the main rh-Lactoferrin HIV study. Thus, you will not be eligible for this substudy. If you become pregnant during the study, you will be asked to withdraw from the main study and this substudy.

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WHAT ARE THE BENEFITS OF THIS SUBSTUDY?

If you take part in this substudy, there are no anticipated long-term benefits to your health. The rh-lactoferrin treatment may improve intestinal health, but the short duration is unlikely to affect your long-term health.

COMPENSATION

You will be paid \$20 for each rectal swab specimen collection procedure that you undergo. This will occur up to 4 times during the study. You may receive a total of \$80.00 for participating in the substudy. Study investigators may ask for your social security number as part of the monitoring process for this compensation.

WHAT IF THERE ARE NEW FINDINGS?

We will not be analyzing data during the study. However, if during the course of this research study, there are significant new findings discovered which might influence your willingness to continue, the researchers will inform you of those developments. You may request your own results after the study by contacting the research investigators.

WHAT IF YOU DON'T WANT TO BE IN THE SUBSTUDY ANY LONGER?

Like your participation in the main research study, the substudy is completely voluntary. You may participate in the main research study even if you decline to participate in the substudy. If you enroll in this substudy, you may decide to stop participating at any time. Withdrawing from this substudy will not affect the benefits of your regular medical care.

CAN YOUR STUDY PARTICIPATION BE STOPPED WITHOUT YOUR CONSENT?

You may be taken off the substudy before the end of the substudy if study investigators or your doctor recommend this. If you are withdrawn from the main study you will also be taken off of this substudy.

WHAT OTHER CHOICES DO YOU HAVE BESIDES THIS SUBSTUDY?

An alternative is not to participate in the substudy.

WHAT ARE THE COSTS TO YOU?

During the study, you, your insurance company, or some other third-party payer must pay for all other medicines, including HIV medicines not paid by the study and medicines needed to prevent or treat other illnesses. We will provide all clinical and professional services, lab work, and other tests that are part of this study and not part of your regular care at no cost to you.

HOW IS YOUR PRIVACY PROTECTED?

Any information that could be used to identify you will be treated in strict confidence to the extent allowed by law. Nevertheless, some uses and disclosures of your information are

APPENDIX A2: INFORMED CONSENT TEMPLATE (MICROBIOME SUBSTUDY)

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necessary to conduct the study. If you agree to be part of this study, you will also be allowing the uses and disclosures of your private health information as needed for the purposes of this study as described in this consent.

“Private health information” means information that identifies you and is collected:

- during this study;
- from your past and current medical records maintained by your regular health care providers (including, if applicable, HCMC), to the extent the information is relevant to this study or to your eligibility for this study; or
- from any payment records relating to items or services furnished to you during this study.

By signing this consent, you are agreeing that your private health information may be disclosed to and used by:

- the doctors and other health care providers involved in this study;
 - their staff;
 - the research center (Minneapolis Medical Research Foundation);
 - members of the HCMC Human Subjects Research Committee/Institutional Review Board;
 - the sponsor of this study and its agents; and
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- Your clinical research labs may be entered into the electronic medical record (EPIC) at Hennepin County Medical Center. These lab results may be viewed by other providers and allied health professionals.

The findings of this study may be used for scientific meetings, written reports, and publications, but no information that could be used to identify you will be disclosed for these purposes. Once your private health information has been disclosed to a third party, federal privacy laws may no longer protect it from re-disclosure. However, anyone obtaining access to your private health information under this consent must agree to protect your information as required by this consent.

This consent to use your private health information as described above does not expire. However, if you later change your mind, you can revoke this consent by writing to Dr. Jason Baker saying that you no longer wish to allow your private health information to be used for this study. If you revoke your consent, you may no longer be able to participate in the study. Moreover, we cannot undo uses or disclosures of your private health information that have already taken place in reliance on your

PLEASE NOTE:

In the event of a positive result for Hepatitis B or C, reporting of the results to the Minnesota the Department of Health is required.

APPENDIX A2: INFORMED CONSENT TEMPLATE (MICROBIOME SUBSTUDY)

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WHAT IF YOU ARE INJURED?

If you are injured as a result of being in this study, you will be given immediate treatment. The cost for treatment will be charged to you or your insurance company. There is no program through this institution to compensate participants who have research related injuries. You will not be giving up any of your legal rights by signing this consent.

WHAT IF YOU HAVE PROBLEMS OR QUESTIONS?

If you ever have questions or in the case of research-related injuries, you should contact:

Jason Baker, MD, MD

701 Park Ave South; Mail Code G5

Minneapolis, MN 55415

612-873-2705

OR

Rachel A. Prosser, PhD, RN, CNP

701 Park Ave South; Mail Code O1.326

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If you have questions about research subject's rights you can contact:

Dr Fred Langendorf, Chairman, Human Subjects Research Committee

701 Park Ave., S9.115

Minneapolis, MN 55415

Phone: 612-873-6882

RESEARCH STUDY REGISTRY

A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as required by U.S. Law. This Website will not include information that can identify you. At most, the Website will include a summary of the results. You can search this Website at anytime.

APPENDIX A2: INFORMED CONSENT TEMPLATE (MICROBIOME SUBSTUDY)

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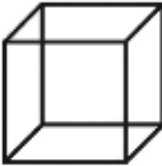
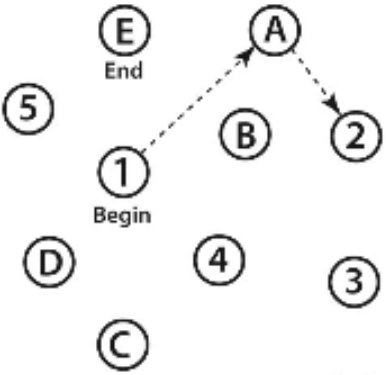



SIGNATURE PAGE FOR CONSENT TO PARTICIPATE IN THE RH-LACTOFERRIN HIV STUDY

I have read this consent form, had the opportunity to ask questions and have received answers to any questions I have asked. I willingly give my consent to participate in this study, and authorize the use and disclosure of my health information as described in this form. By signing this consent form I do not give up any of my legal rights. Upon signing this form I will be given a signed copy of the form for my records.

If you have read the informed consent (or if you have had it explained to you) and understand the information, and you voluntarily agree to join this study, please sign your name below.

_____ Participant's name (typed or printed)	_____ Participant's signature	_____ Date
_____ Signature of person obtaining consent		_____ Date

APPENDIX B: Montreal Cognitive Assessment (MOCA)

MONTREAL COGNITIVE ASSESSMENT (MOCA) Version 7.1 Original Version				NAME : Education : Sex :		Date of birth : DATE :	
VISUOSPATIAL / EXECUTIVE				 Copy cube		Draw CLOCK (Ten past eleven) (13 points)	
 [] []				[] [] [] Contour Numbers Hands		___/5	
NAMING							
 []		 []		 []		___/3	
MEMORY							
Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.				FACE VELVET CHURCH DAISY RED		No points	
1st trial							
2nd trial							
ATTENTION							
Read list of digits (1 digit/ sec.).				Subject has to repeat them in the forward order [] 2 1 8 5 4 Subject has to repeat them in the backward order [] 7 4 2			
Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors				[] FBACMNAAJ KLBAFAKDEAAA JAMOF AAB			
Serial 7 subtraction starting at 100 [] 93 [] 86 [] 79 [] 72 [] 65				4 or 5 correct subtractions: 3 pts, 2 or 3 correct: 2 pts, 1 correct: 1 pt, 0 correct: 0 pt			
LANGUAGE							
Repeat : I only know that John is the one to help today. [] The cat always hid under the couch when dogs were in the room. []				___/2			
Fluency / Name maximum number of words in one minute that begin with the letter F [] ____ (N ≥ 11 words)				___/1			
ABSTRACTION							
Similarity between e.g. banana - orange = fruit [] train - bicycle [] watch - ruler				___/2			
DELAYED RECALL							
Has to recall words WITH NO CUE		FACE []	VELVET []	CHURCH []	DAISY []	RED []	Points for UNCUED recall only
Optional							
Category cue Multiple choice cue							
ORIENTATION							
[] Date [] Month [] Year [] Day [] Place [] City				___/6			
© Z.Nasreddine MD				www.mocatest.org		Normal ≥ 26 / 30	
Administered by: _____				TOTAL		___/30	
				Add 1 point if ≤ 12 yr edu			